Structural and Mechanistic Studies on WhiB7 in Antibiotic Resistance of Mycobacteria

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Background

Mycobacterium tuberculosis (Mtb)

- Pathogenic bacteria that causes tuberculosis (TB), a disease that affects 1/3 of the world population (CDC, 2016).
- Difficult to treat due to:
  - Intrinsic resistance of the cell membrane.
  - A complex regulatory system that responds and confers resistance to many antibiotics.

WhiB7

- An iron sulfur cluster-bound transcriptional regulator well conserved in mycobacteria pathogens, only found in actinobacteria.
- Responds to a variety of clinically relevant antibiotics, such as Spectomycin and kanamycin (1).
- Responds to other redox stress conditions.

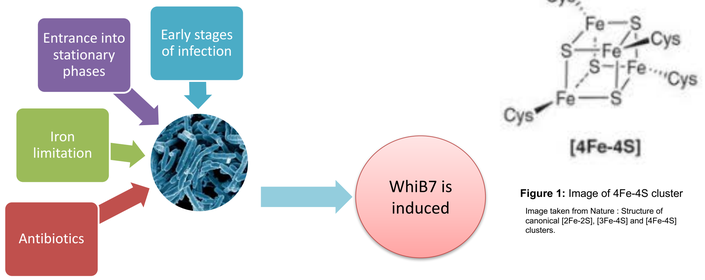


Image taken from NIH NIAID : Tuberculosis(TB)

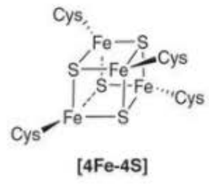


Figure 1: Image of 4Fe-4S cluster

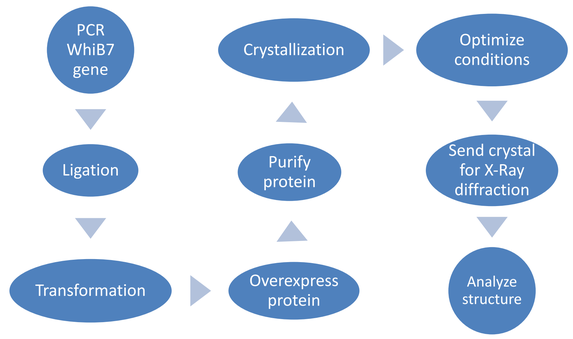
Image taken from Nature : Structure of canonical [2Fe-2S], [3Fe-4S] and [4Fe-4S] clusters.

- Binds to SigA, a primary sigma factors that enables the binding of RNA polymerase to specific promoter regions.
- While we know WhiB7 plays a role during stressful situations, we do not know what specifically signals the whiB7 gene to start transcription (2).

Significance & Hypothesis

- The purpose of this study is to understand the mechanism and structure of WhiB7 to provide tools against antibiotic resistance in TB.
- **Hypothesis:** A stress condition such as antibiotic treatment causes an oxidative state shift within the cell which causes a redox dependent confirmation change in WhiB7 and thus activates WhiB7.

Materials & Methods



Results

Overexpression and purification of SigA-WhiB7 with NiN-TA and gel filtration column:

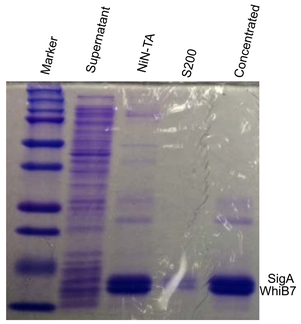


Figure 2: Co-Purification of *Mtb*-WhiB7-SigA

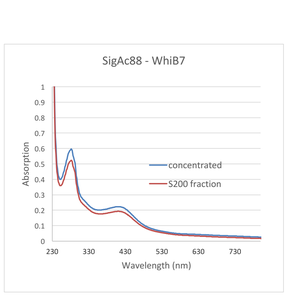


Figure 3: Absorbance reading for purification of *Mtb*-WhiB7

Overexpression and purification of *Msm*-WhiB7 under aerobic and anaerobic conditions:

- Absorbance at 410nm is indicative of the presence of 4Fe-4S cluster.
- Purification under aerobic conditions has a lower 410 reading compare to the anaerobic purification.

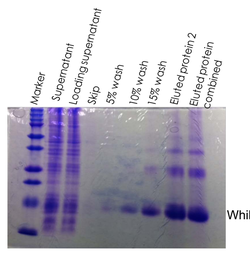


Figure 4: Absorbance reading for purification of *Msm*-WhiB7 in aerobic conditions

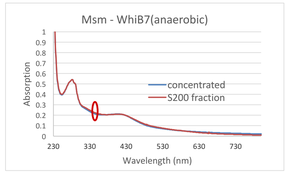


Figure 5: Absorbance reading for purification of *Msm*-WhiB7 in anaerobic conditions

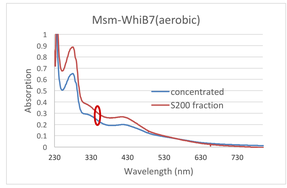


Figure 6: SDS page for purification of *Msm*-WhiB7 in anaerobic conditions

Stability of WhiB7 under anaerobic and oxidizing conditions:

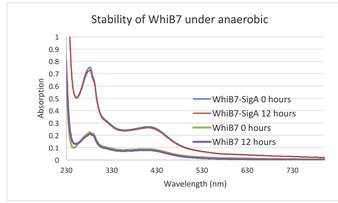


Figure 7: Absorbance spectra of stability test under reductive conditions over a period of 12 hours

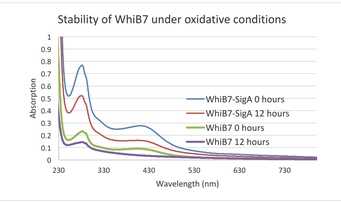


Figure 8: Absorbance spectra of stability test under oxidative conditions over a period of 12 hours

- No significant loss of protein under reductive conditions.
- Similar loss of protein for *Msm*-WhiB7 and SigA-WhiB7 under oxidative conditions after 12 hours.
- 410nm reading intact after 12 hours under anaerobic for both samples.
- 410 reading decrease under oxidative conditions for both sample as is expected.

Conclusions & Discussion

- To conclude with we managed to purify Mtb-WhiB7-SigA to a quality and quantity for crystallization screening.
- WhiB7 highly unstable under oxidizing conditions.

Future Work

- Crystallization screening of WhiB7.
- Test the redox reaction of WhiB7 with redox active molecules.

Acknowledgements

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MCNAIR SCHOLARS PROGRAM

References

1) Morris, R. P., L. Nguyen, J. Gatfield, K. Visconti, K. Nguyen, D. Schnappinger, S. Ehrt, Y. Liu, L. Heifets, J. Pieters, G. Schoolnik, and C. J. Thompson. "Ancestral Antibiotic Resistance in Mycobacterium Tuberculosis." Proceedings of the National Academy of Sciences 102.34 (2005): 12200-2205.

2) Burian, J., S. Ramon-Garcia, G. Sweet, A. Gomez-Velasco, Y. Av-Gay, and C. J. Thompson. "The Mycobacterial Transcriptional Regulator WhiB7 Gene Links Redox Homeostasis and Intrinsic Antibiotic Resistance." Journal of Biological Chemistry 287.1 (2011): 299-310. Web. 16 May 2017.